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Regulation of Growth, Antioxidants and Sugar Metabolism in Rice (*Oryza sativa* L.) Seedlings by NaCl and Its Reversal by Silicon

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Abstract

The effect of NaCl with or without silicon on the growth and metabolism in rice seedlings cv. MTU1010 was studied. In these seedlings, the oxidative stress has been observed with NaCl treatments and the levels of proline, H_2O_2 and malondialdehyde contents were increased whereas catalase activity was decreased. NaCl exposure at 25 mM, 50 mM and 100 mM concentrations in the test seedlings resulted in an increase in both reducing and non-reducing sugar content. There was a decrease in starch contents and the activity of starch phosphorylase was increased. NaCl stress also affected the activities of different carbohydrate metabolizing enzymes. The activities of sucrose synthase and sucrose phosphate synthase were increased, while the activity of acid invertase was decreased. Joint application of silicon with NaCl showed significant alterations on all parameters tested under the purview of NaCl treatment alone leading to better growth and metabolism in rice seedlings. Thus the use of silicon enriched fertilizers may help to grow healthy rice plants in NaCl rich soil.

Keywords

Rice, NaCl, Silicon, Antioxidant, Sugar Metabolism

1. Introduction

Soil salinity is a major problem for agriculture throughout the world. Soil salinization is the enrichment of salts, mainly sodium chloride or sodium sulphate, at or near the soil surface. It is one of the most widespread stress

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hazards and occurs in arid and semi-arid lands, but in eastern India this also exists in subhumid to humid and coastal lands. About seven million hectares in the country (0.840 hectares in West Bengal) have either gone out of cultivation or this area produces low yields of crops.

Salt stress reduces the free energy of soil water available to plants and results in negative water potential in soils. This drop in water potential is accompanied by specific ion toxicities and deficiencies, retardation of water uptake and nutritional imbalance in plants [1]. Improvement in salt tolerance of crop plants remains elusive, due to the fact that salinity affects almost every aspect of the physiology and biochemistry of plants [2] either in whole plant or in cellular levels [3]. Soil salinity affects plants through osmotic effects, ion-specific effects, and oxidative stress [4]. The effect of salinity stress in plants is mediated at least in part by an enhanced generation of active oxygen species, especially in chloroplasts and mitochondria which cause lipid peroxidation and membrane injury, protein degradation and enzyme inactivation. Plants have developed a complex antioxidant system which mitigates and repairs the damage initiated by reactive oxygen species, toward enzyme synthesis to protect the cellular and subcellular systems from the cytotoxic effects of these active oxy-free radicals.

Silicon, the second most abundant element on earth, second only to oxygen [5], is ever-present in plants [6], in quantities equal or more than many macro nutrients [7]. Si is found as silicic acid (H_4SiO_4) in the soil solution [7], and when absorbed, it is transported to shoots and polymerises into solid amorphous silica ($SiO_2 \cdot nH_2O$), called opal, found mainly in cell walls [6]. Si has a well documented ability to ameliorate stresses associated with salinity in plants, specifically, stresses associated with high levels of NaCl. This is found in a wide range of plant species, including tomato [8], cucumber [9], grapevine [10] and sugarcane [11]. Si was reported to reduce the hazardous effects of various abiotic and biotic stresses including salt stress, metal toxicity, drought stress, radiation damage, various pests and diseases caused by both fungi and bacteria, nutrients imbalance, high temperature and freezing [12].

Carbohydrates produced by photosynthetic tissues during the different growth stages is either transported to other organs as soluble sugars, or accumulated in leaves as soluble sugars and starch. The ability of plants to recover from most abiotic stresses normally increases with increasing concentrations of photosynthetic assimilates in plant tissues during or after stress [13] [14]. Sugar accumulation under stress contributes to osmoregulation and provides protection of biomolecules. Accumulation of soluble carbohydrates and starch under normal conditions before the stress commonly constitutes the main resources for plants to supply energy during stress condition, as well as during recovery [15]. Higher concentrations of carbohydrates in plant tissue are one of the major adaptive mechanisms as observed under submergence [16] [17]. Starch accumulates in the leaves as a temporary reserve form of carbon and then is stored in the cereal grains. Sucrose is the major form of translocated carbon [18]. Among the other significant salt stress consequences are changes in the contents of non-reducing and reducing sugars as well as in sucrose synthase (SS; EC 2.4.1.13), sucrose phosphate synthase (SPS; EC 2.4.1.14), acid invertase (EC 3.2.1.26) activities and starch phosphorylase (EC 2.4.1.1) [19] [20]. Starch phosphorylase degrades starch by incorporating phosphate at the nonreducing end [21].

Rice is one of the most important food crops, serving as the staple food for over one-third of the world's population [22]. It is one of the most widely grown crops in coastal areas frequently inundated with sea water during high tidal period [23]. Salinity is one of the important physical factors that influence rice production. At present, salinity is the second most widespread soil problem in rice growing countries after drought and is considered as a serious constraint to increased rice production worldwide [24]. The seedling stage is one of the most sensitive stages to salt stress in rice, and studies on salt tolerance during this stage could probably provide insights for enhancing tolerance throughout the plant life cycle [25]. The present investigation was undertaken to examine the influence of NaCl on the metabolic status of starch, sugars and different sugar metabolizing enzymes in germinating rice seedlings. A combined application of sodium chloride with silicon was tried to combat the serious problem of NaCl toxicity and to provide a possible method of salt tolerance, with an aim to improve growth and metabolism of rice plants under saline soil conditions.

2. Plant Materials and NaCl Treatments

Rice (*Oryza sativa* L.) seeds cv. MTU 1010 obtained from the State Rice Research Station, Chinsurah, Hooghly, West Bengal were surface sterilized with sodium hypochlorite (5%, v/v) and washed thoroughly. About 50 seeds for each treatment were spread over in petridishes (φ 10 cm) lined with filter papers. The seeds were kept in dark and humid conditions for 48 hours in a germinator at 30˚C ± 2˚C. Now the germinating seeds were exposed to

25, 50, 100 mM concentration of sodium chloride (NaCl: Merck, India) solutions (w/v) wih or without 2 mM Sodium meta Silicate (Na_2O_3Si, $9H_2O$: Loba-chemie, India) solution (w/v) and exposed to 16 hours photoperiod (260 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PFD) for 21 days in presence of modified Hoagland solution. The seedlings were harvested after 21 days for the following studies.

2.1. Morphological Studies

After twenty one days, NaCl induced damaging effects were observed and the relevant root lengths and shoot lengths of growing rice seedlings were measured. The seedlings were harvested, weighed in equal amount for each set and stored at −40˚C for further biochemical studies.

2.2. Malondialdehyde Contents (Lipid Peroxidation)

Lipid peroxidation in terms of malondialdehyde (MDA) was determined to assess the membrane damage in rice seedlings. For the measurement of lipid peroxidation, TBA test was used to measure MDA level as an end product of lipid peroxidation [26]. 1 g tissue was homogenized in 4 ml of 1% TCA solution (w/v) and centrifuged at 10,000 g for 10 min. The supernatant was mixed with 1 ml of 0.5% TBA in 20% TCA (w/v). The mixture was incubated in boiling water for 30 min and the reaction was stopped by placing the tubes in an ice bath. The samples were re-centrifuged at 10,000 g for 5 min and the absorbance values of the supernatants were measured at 532 nm. The values for non-specific absorption at 600 nm were substracted. The amount of MDA-TBA complex present was calculated using an extinction coefficient (ε) of 155 $mM^{-1} \cdot cm^{-1}$ and expressed as $\mu M \cdot g^{-1}$ fw.

2.3. Proline Contents

1 g tissue of rice seedlings was extracted with 5 ml of 0.1 M sulphosalicylic acid and centrifuged at 5000 g for 30 min [27]. To 2 ml of supernatant, 5 ml glacial acetic acid and 5 ml of 140 mM acid ninhydrin were added and shaken vigorously. The mixture was heated in a boiling water bath and after cooling, the mixture was extracted in 10 ml of toluene in a separating funnel and aqueous layer was discarded. The absorbance of the mixture was measured at 520 nm. The proline content was calculated from standard curve and expressed as $\mu g \cdot g^{-1}$ fw.

2.4. H_2O_2 Contents

The H_2O_2 content from rice seedlings was measured according to [28]. 1 g tissue was extracted with 5 ml of TCA (0.1%, w/v) at 48˚C and homogenate was centrifuged at 12,000 g for 15 min. To 0.5 ml supernatant, 0.5 ml of 0.05 M sodium phosphate buffer (pH 7.0) and 1 ml of 1M potassium iodide solution were added. The absorption of the mixture was measured at 390 nm. The H_2O_2 content was determined using an extinction coefficient (ε) of 0.28 $\mu M^{-1} \cdot cm^{-1}$ and expressed as $\mu M \cdot g^{-1}$ fw.

2.5. Assay of Catalase Activity

Catalase activity was determined as the amount of potassium permanganate ($KMnO_4$) consumed in terms of H_2O_2 [29]. Enzyme extraction procedure was carried out at 4˚C. 1 g plant sample was homogenized in 5.0 ml of pre-chilled 0.1 M sodium phosphate buffer (pH 7.0). The homogenate was centrifuged at 12,000 g for 20 min and supernatant was used to assay the enzyme activity. The reaction mixture contained 0.5 ml of 0.1 M sodium phosphate buffer (pH 7.0), 1 ml of 3% H_2O_2 (v/v) and 1 ml of enzyme extract. After incubation, the reaction was stopped by adding 3 ml of 10% H_2SO_4 (v/v). The residual H_2O_2 was titrated against 0.02(N) $KMnO_4$. The enzyme activity was expressed in terms of mg H_2O_2 decomposed mg^{-1} protein min^{-1}.

2.6. Estimation of Reducing and Non-Reducing Sugar

Estimation of reducing sugar was done by the method of [30]. 1 gm of plant material was crushed with 80% ethanol and centrifuged at 2000 rpm for 20 minutes. To 1.0 ml of alcoholic extract, DNSA reagent was added and boiled in water bath for 5 min. The absorbance was measured at 515 nm using Hitachi-2000 spectrophotometer. From a standard curve of glucose, the quantity of reducing sugar was calculated and expressed as $mg \cdot g^{-1}$ fw. The amount of non-reducing sugar was measured by substracting the value of reducing sugar from the value

of total soluble sugar.

2.7. Starch Contents

Estimation of starch was done according to [31]. The residual mass, obtained after centrifugation for the extraction of total soluble sugar was suspended in distilled water followed by the addition of perchloric acid. After stirring, the mixture was centrifuged at 2000 rpm for 20 minutes. The supernatant was collected and poured in conical flasks and the total volume was made up to 100 ml by adding distilled water for each set. Then 1.0 ml of filtrate from each set was taken and starch content measured following the same procedure as the total soluble sugar. Quantity of starch was calculated in terms of glucose and factor 0.9 was used to convert the values of glucose to starch. The quantity of starch was expressed in $mg \cdot g^{-1}$ fw.

2.8. Assay of Acid Invertase Activity

Acid invertase was assayed according to [32]. Root and shoot samples were homogenized in 10mM sodium acetate buffer (pH 4.6) containing 3.3 mM $MgCl_2$, 1 mM EDTA, and 1 mM PMSF. The homogenates were centrifuged at 10,000 rpm and 4˚C for 20 minutes. The assay mixture contained 10 mM sodium acetate buffer (pH 4.6), 0.4 M sucrose, and enzyme extract to make the total volume up to 1.0 ml. After incubation at 30˚C for 30 minutes, the reaction was terminated with the addition of 0.5 M Na_2HPO_4. The resulting reducing sugars were estimated according to [33] [34]. Invertase activity was expressed as µmol sucrose hydrolysed mg^{-1} protein min^{-1}.

2.9. Assay of Starch Phosphorylase Activity

For determination of starch phosphorylase activity [35], plant materials from each treatment were homogenized in 50 mM citrate buffer (pH 6.0) containing 1 mM EDTA, 5 mM β-mercaptoethanol and 1mM PMSF and centrifuged at 10,000 rpm for 20 minutes at 4˚C. The assay mixture contained 50 mM citrate buffer (pH 6.0), 5% soluble starch (w/v), 0.1 mM glucose-1-phosphate and enzyme extract to make the total volume upto 4.0 ml. The reaction was stopped after 10 minutes by adding 5% TCA. The mixture was centrifuged and phosphorus content in the supernatant was estimated according to [36]. The enzyme activity was calculated as µmol of Pi liberated mg^{-1} protein min^{-1}.

2.10. Assay of Sucrose Synthase and Sucrose Phosphate Synthase Activity

For the assay of sucrose phosphate synthase (SPS) and sucrose synthase (SS), the respective tissues were extracted following the method of [37] and assayed according to [38]. Plant samples were extracted in 50 mM HEPES-NaOH buffer (pH 7.5) containing 5 mM $MgCl_2$, 1 mM EDTA, 2.5 mM DTT and 0.05% (v/v) Triton X-100, at 4˚C, centrifuged at 10,000 rpm and 4˚C for 10 minutes. Assay mixture for SPS contained 50 mM HEPES-NaOH buffer (pH 7.5), 15 mM $MgCl_2$, 25 mM fructose-6-phosphate, 25 mM glucose-6-phosphate, 25 mM UDP-glucose and enzyme extract. Mixtures were incubated for 30 min at 37˚C and reaction was terminated with the addition of 30% KOH. The reaction mixture for SS assay was similar to SPS assay but it contained 25 mM fructose instead of fructose-6-phosphate and was devoid of glucose-6-phosphate. The sucrose hydrolysed during SS catalyzed reaction and sucrose formed during SPS catalyzed reaction were estimated according to [39]. The enzyme activities were expressed as µmol sucrose hydrolysed or formed mg^{-1} protein min^{-1} respectively.

2.11. Statistical Analysis

The experiments were carried out in a completely randomized design (CRD) with three replicates; each replica comprising a single petridish containing an average of 50 seeds. The data and significant differences among the mean values were compared by descriptive statistics (±SE).

3. Results

3.1. Effect on Growth

The lengths of shoot and root of twenty one days old growing rice (cv. MTU 1010) seedlings were affected by

NaCl treatment. In roots, there was a decrease in length by 8%, 33% and 51% under 25 mM, 50 mM and 100 mM NaCl treatment respectively over water control. In roots, there was an average decrease of about 20% in length when seedlings were jointly treated with 50 mM and 100 mM NaCl and 2 mM silicon whereas it increased to 21% on 25 mM NaCl along with silicon treatment (**Figure 1**)

The shoots of the samples exhibited a decrease in length by about 23%, 26% and 55% under 25 mM, 50 mM and 100 mM NaCl treatment respectively over water control. During joint application of NaCl of above concentrations with 2 mM silicon, there was a decrease in shoot length on an average by about 21% which is much lower in respect of seedlings treated with NaCl alone (**Figure 2** and **Figure 3**).

3.2. Effect on Antioxidant Enzyme Activities

Catalase

The activity of catalase in the roots and shoots of rice seedlings treated with 25 mM, 50 mM, 100 mM NaCl decreased in an average by about 70% and 54%, respectively, over water control. Under joint application of NaCl

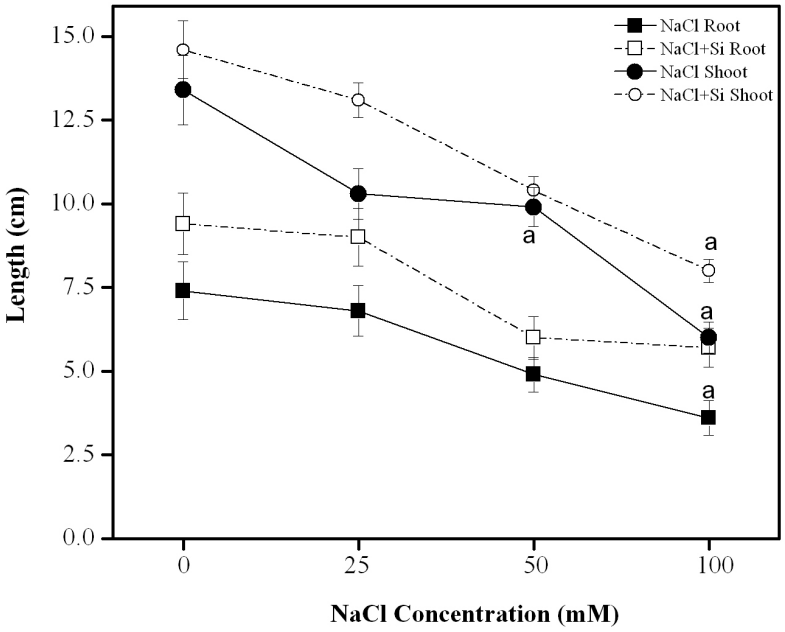


Figure 1. Effect of sodium chloride and/or silicon on root and shoot length of twenty-one days old rice (cv. MTU 1010) seedlings. Each data point is the mean ± SE with 3 replicates. [a]indicates statistically significant at $P \leq 0.05$ respectively as compared to water control.

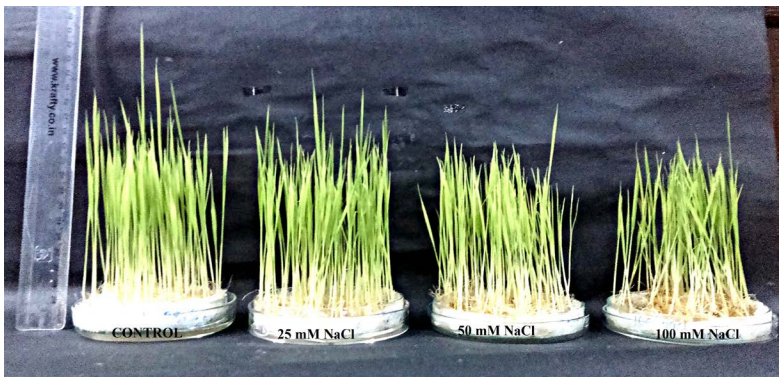


Figure 2. Effect of NaCl on relative root and shoot length of twenty-one days old rice (cv. MTU 1010) seedlings.

and Si, the rate of decrease in enzyme activity in an average were 34% and 40% in root and shoot samples respectively (**Figure 4**).

3.3. Effect on Oxidative Stress Markers

3.3.1. Proline Contents

NaCl treatments considerably increased the proline contents in roots and shoots of rice seedlings. The rate of increment was more pronounced in shoots than in roots. Proline contents were increased by about 67%, 118% and 153% respectively in roots and by about 360%, 426% and 556% respectively in shoots under 25 mM, 50 mM and 100 mM NaCl treatment respectively. Under joint application of NaCl and Si, proline contents increased in an average of about 69% and 305% over water control in roots and shoots respectively (**Figure 5**).

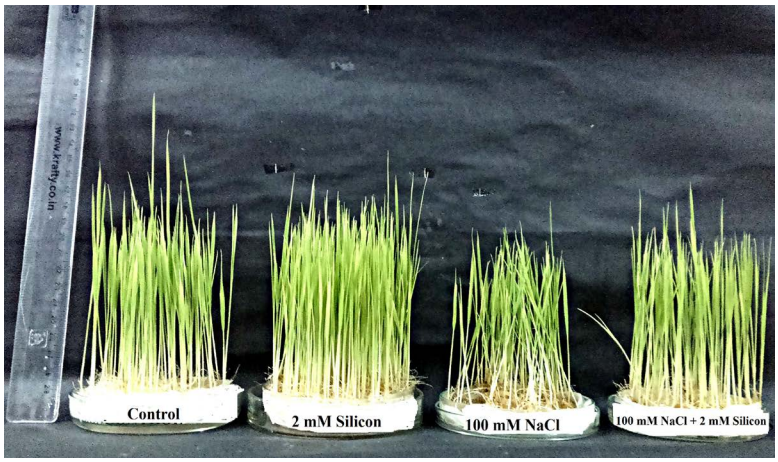


Figure 3. Effect of sodium chloride and/or silicon on root and shoot length of twenty one days old rice (cv. MTU 1010) seedlings.

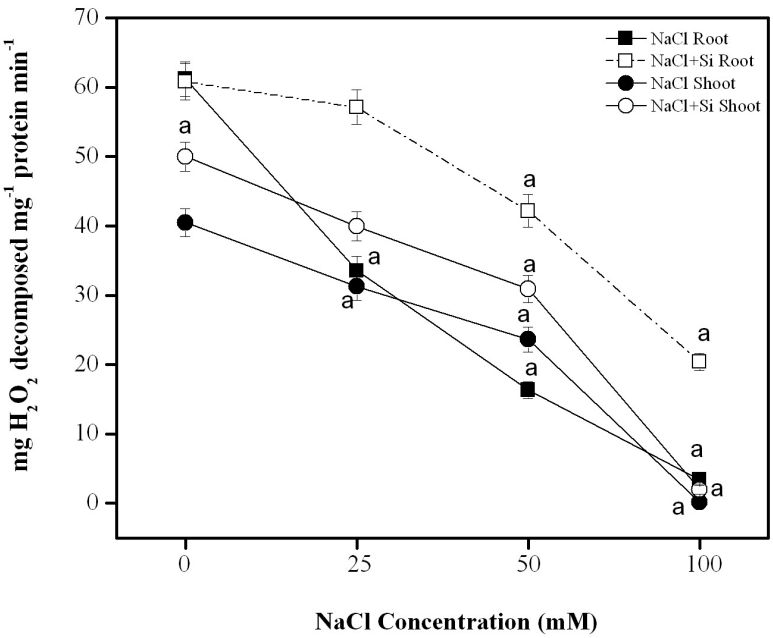


Figure 4. Effect of sodium chloride and/or silicon on the activity of catalase in root and shoot of twenty one days old rice (cv. MTU 1010) seedlings. Each data point is the mean ±SE with three replicates. [a]indicates statistically significant at $P \leq 0.05$ respectively as compared to water control.

3.3.2. MDA and H_2O_2 Content

Enhanced rate of lipid peroxidation was recorded as indicated by gradually increase in malondialdehyde (MDA) contents in the test seedlings exposed to NaCl. The MDA contents increased in all NaCl treated seedlings amounting to an average of about 133% in roots and 47% in shoots over water control. The elevated levels of MDA reduced from 133% to 83% and 47% to 10% in roots and shoots respectively under joint application of NaCl and Si (**Figure 6(A)**). Similarly H_2O_2 contents in the NaCl treated test seedlings were increased to an average of about 84% in roots and 41% in shoots, compared to water control. While joint application of NaCl with Si, reduced the level of H_2O_2 from 84% to 65% in roots and 41% to 29% in shoots in an average when compared to water control (**Figure 6(B)**).

3.4. Effect on Sucrose Synthesizing Enzyme Activity

Sucrose Phosphate Synthase

The activity of sucrose phosphate synthase (SPS) increased in both roots and shoots of the test seedlings. In

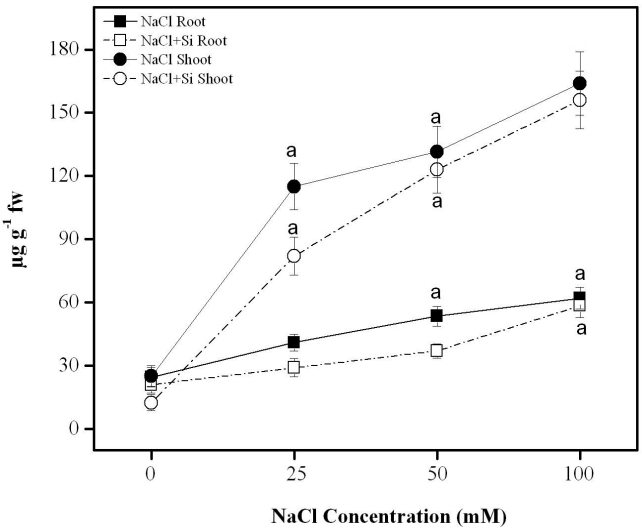
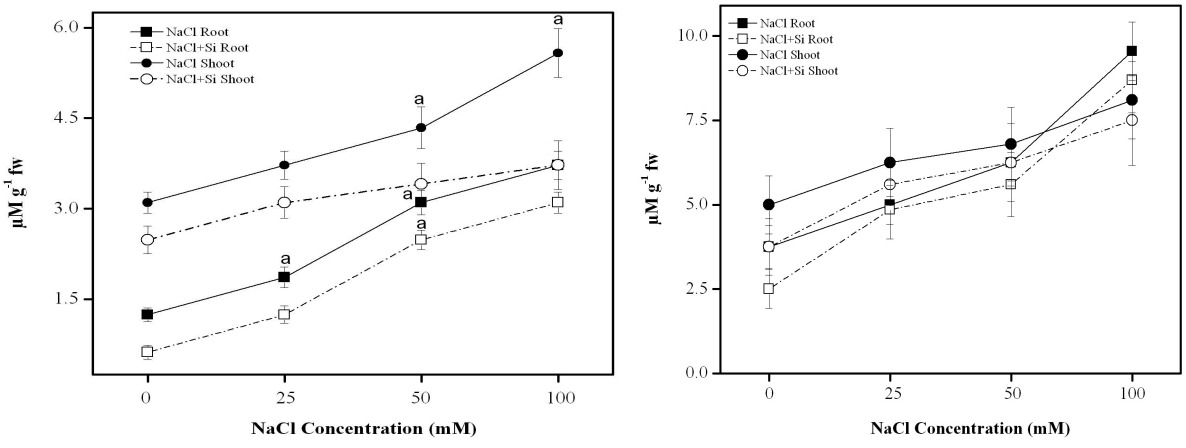


Figure 5. Effect of sodium chloride and/or silicon on proline contents in root and shoot of twenty-one days old rice (cv. MTU 1010) seedlings. Each data point is the mean ±SE with 3 replicates. [a]indicates statistically significant at $P \leq 0.05$ respectively as compared to water control.



(A) (B)

Figure 6. Effect of sodium chloride and/or silicon on MDA (A) and H_2O_2 (B) contents in root and shoot of twenty-one days old rice (cv. MTU 1010) seedlings. Each data point is the mean ±SE with 3 replicates. [a]indicates statistically significant at $P \leq 0.05$ respectively as compared to water control.

roots, about 68%, 106% and 189% increase in enzyme activity was recorded at 25 mM, 50 mM and 100 mM NaCl treatments respectively while in shoots, the SPS activity was increased by about 47%, 79% and 121% over water control at the same levels of NaCl treatment (**Figure 7**). On the contrary, application of 2 mM silicon along with 25 mM, 50 mM and 100 mM NaCl led to increase in enzyme activity to about 30% 65% and 145% respectively in roots and by about 29%, 42% and 72% respectively in shoots.

3.5. Effect on Sucrose Degrading Enzyme Activities

3.5.1. Sucrose Synthase

The effect of NaCl on rice seedlings showed increased activity of sucrose synthase in both roots and shoots. In roots, the activity was increased by 2%, 29% and 63% at 25 mM, 50 mM and 100 mM NaCl treatments respectively, while in shoots about 8%, 19% and 31% increase in enzyme activity was recorded under same concentrations of NaCl. Co-application of silicon along with same doses of NaCl, the sucrose synthase activity was altered both in root and shoot samples. In root, the enzyme activity slightly decreased during silicon and NaCl treatments, but increased by about 6% in 100 mM NaCl treatment. In shoot, said enzyme activity increased by silicon and NaCl application on an average by 8% over water control (**Figure 8(A)**).

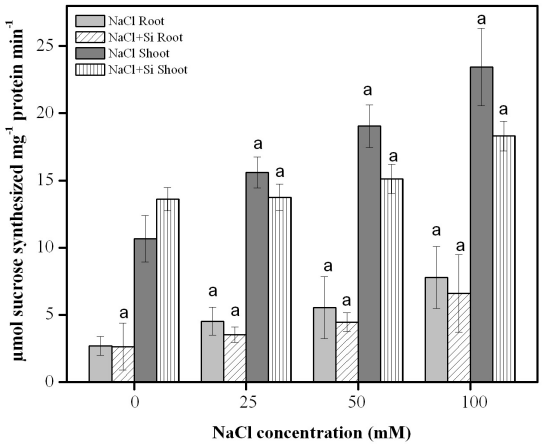


Figure 7. Effect of sodium chloride and/or silicon on the activity of sucrose phosphate synthase (SPS) in root and shoot of twenty-one days old rice (cv. MTU 1010) seedlings. Each data point is the mean ±SE with 3 replicates. [a]indicates statistically significant at $P \leq 0.05$ respectively as compared to water control.

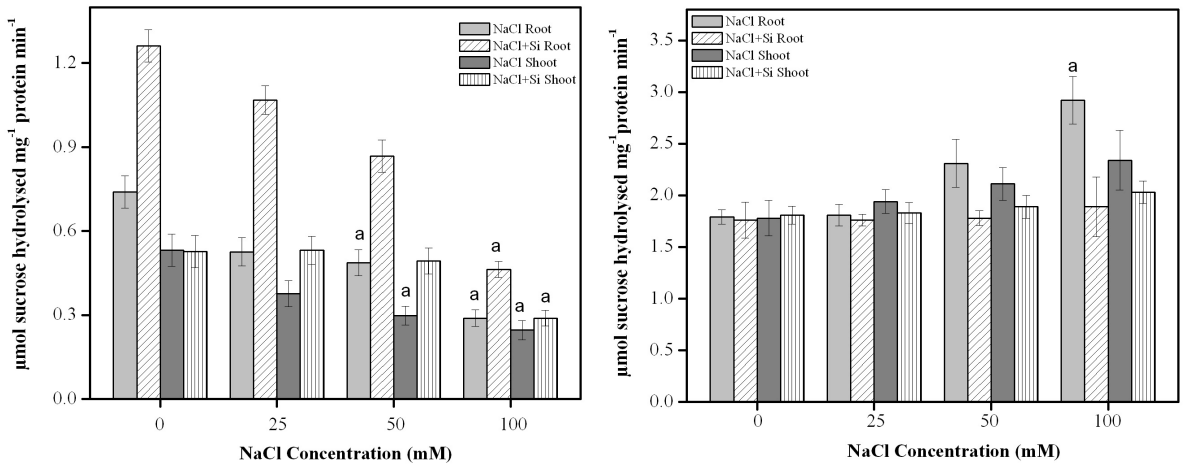


Figure 8. Effect of sodium chloride and/or silicon on the activities of sucrose synthase (SS) (A) and acid invertase (B) in root and shoot of twenty-one days old rice (cv. MTU 1010) seedlings. Each data point is the mean ±SE with 3 replicates. [a]indicates statistically significant at $P \leq 0.05$ respectively as compared to water control.

3.5.2. Acid Invertase

Rice seedlings showed considerable decrease in activity of acid invertase in both roots and shoots due to NaCl treatment. In shoots, about 29%, 43% and 53% decrease in enzyme activity was observed in 25 mM, 50 mM and 100 mM NaCl treatments respectively, while in roots the said level of decrease was about 28%, 34% and 60% over water control (**Figure 8(B)**). However, joint application of silicon and NaCl altered the acid invertase activity significantly in the test seedlings over NaCl treatment alone. In shoots, the rate of decrease in enzyme activity was narrowed down, on an average, to about 18% over water control. While in roots, the increase in enzyme activity was about 44% and 17% under 25 mM and 50 mM NaCl treatment respectively but decreased by about 37% under 100 mM NaCl treatment (**Figure 8(B)**).

3.6. Effect on Starch Hydrolyzing Enzymes Activity

Starch Phosphorylase Activity

The activity of starch phosphorylase in the shoots of rice seedlings was increased by NaCl treatment. When seedlings were treated with 25 mM, 50 mM and 100 mM NaCl, there was an increase in enzyme activity by about 47%, 76% and 106% respectively over water control. During joint application of NaCl with silicate the rate of increase in enzyme activity was much less, by about 2% and 31% in 50 mM and 100 mM NaCl treatments respectively whereas decrease in enzyme activity by 18% in 25 mM NaCl treatment over water control. In the roots of the test seedlings treated with 25 mM, 50 mM and 100 mM NaCl there was an increase in starch phosphorylase activity by about 107%, 171% and 433% respectively over water control. During joint application of NaCl of above concentrations with 2 mM silicate the rate of increase in enzyme activity was much less, by about 9%, 80% and 199% over water control (**Figure 9**).

3.7. Effect on Reducing Sugar Contents

The reducing sugar contents in NaCl treated rice seedlings were increased with respect to water control. On an average, in shoots about 17% and in roots about 37% increments were recorded. Co-application of silicon with NaCl altered the level of reducing sugar present in both root and shoot samples in respect of only NaCl treatment. In roots there was an increase on an average of about 19%, in shoots of about 13% in the reducing sugar contents when seedlings were treated with 50 mM and 100 mM NaCl along with silicon where as it decreased to 13% in 25 mM NaCl and silicon treatment (**Figure 10(A)**).

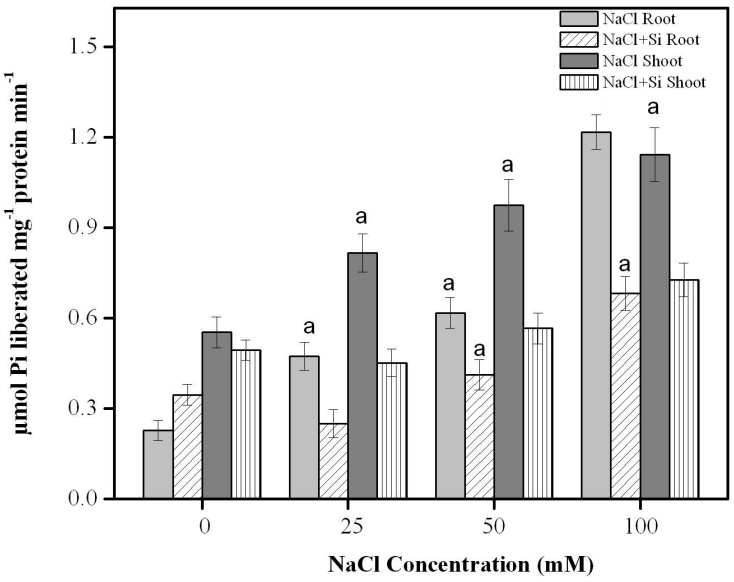


Figure 9. Effect of sodium chloride and/or silicon on the activity of starch phosphorylase in root and shoot of twenty-one days old rice (cv. MTU 1010) seedlings. Each data point is the mean ±SE with 3 replicates. [a]indicates statistically significant at $P \leq 0.05$ respectively as compared to water control.

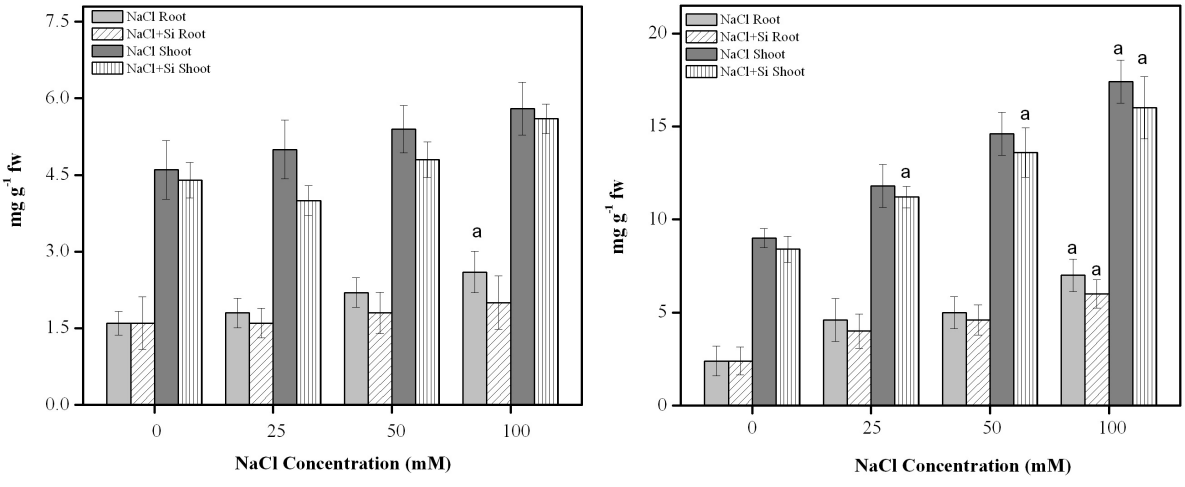


Figure 10. Effect of sodium chloride and/or silicon on reducing sugar (A) and non-reducing sugar (B) contents in root and shoot of twenty one days old rice (cv. MTU 1010) seedlings. Each data point is the mean ±SE with three replicates. [a]indicates statistically significant at $P \leq 0.05$ respectively as compared to water control.

3.8. Effect on Non-Reducing Sugar Contents

The roots and shoots of NaCl treated rice seedlings showed increase level of non reducing sugar contents as compared to water control. In shoot, 25 mM NaCl treatment led to about 31% increase in non-reducing sugar contents while 50 mM and 100 mM treatments increased the said level by about 62% and 93% respectively. During joint application of NaCl with 2 mM silicate the increment in non-reducing sugar contents was much less by about 24%, 51% and 77% over water control. The amount of non reducing sugar in roots was found to be increased by about 91%, 108% and 191% in 25 mM, 50 mM and 100 mM NaCl treated samples respectively. By silicon application along with NaCl, the level of promotion caused by NaCl alone was narrowed down in roots that were about 66%, 91% and 150% in 25 mM, 50 mM and 100 mM NaCl treatment respectively (**Figure 10(B)**).

3.9. Effect on Starch Contents

In both shoot and root of the test seedlings the amount of starch contents was found to be decreased with higher concentrations of NaCl treatment. In shoot, obtained from seedlings treated with 25 mM, 50 mM and 100 mM NaCl about 13%, 33% and 38% decrease in starch contents were recorded whereas, silicon treatment along with NaCl led to about 13% increase, in an average, in starch contents. Likewise, in roots, the starch contents was found to be decreased by 16%, 33% and 50% at 25 mM, 50 mM and 100 mM NaCl treatment respectively. By joint application of silicon and NaCl the amount of starch contents increased by about 17% in 25 mM NaCl treatment but decreased by about 17% and 33% in 50 mM and 100 mM NaCl treatments respectively (**Figure 11**).

4. Discussion

Growth reduction is generally observed in plants exposed to salinity stress. This may be partly due to lower water potential in the cells which, in turn, causes stomatal closure and limits CO_2 assimilation. In the present study, rice seedlings showed reduction in growth of both roots and shoots when exposed to salinity. A marked reduction in growth was reported earlier in rice seedlings exposed to salinity stress [40].

Salt stress significantly increased the H_2O_2 levels and added Si decreased the H_2O_2 levels. This result is in accordance with the observations made by [41]. Since, Singha and Choudhuri (1990) [42] proposed that H_2O_2 accumulation in leaves of cowpea and rice seedlings under salt stress resulted from the decrease in the activity of CAT, H_2O_2 level might have increased due to decreased activity of CAT in seedlings of rice under salt stress.

In many plants, salinity leads to lipid peroxidation implying oxidative damage of cellular membranes and

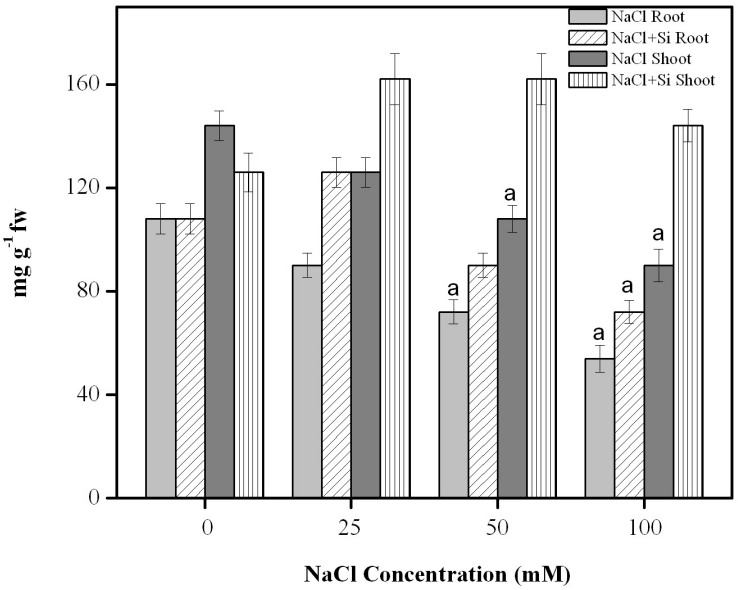


Figure 11. Effect of sodium chloride and/or silicon on starch contents in root and shoot of twenty-one days old rice (cv. MTU 1010) seedlings. Each data point is the mean ±SE with 3 replicates. [a]indicates statistically significant at $P \leq 0.05$ respectively as compared to water control.

their functional perturbations [43]-[45]. In our study NaCl significantly increased the MDA contents in the roots and shoots of the rice seedlings under absence of Si, however, MDA contents was less increased by the addition of Si in presence of NaCl. These results are in accordance with that of [46] and [41]. This study showed that lipid peroxidation induced by NaCl was lower in the Si-treated rice seedlings under salt stress than those under salt stress without Si treatment, which revealed protection of lipid membranes from ROS [47], primarily by more effective scavenging of ROS by means of Si application. Similarly, Si application decreased malondialdehyde and H_2O_2 contents in spinach and tomato plants grown in sodic-B toxic soil [48].

Some reports implicate on the positive correlation of proline accumulation to drought and salt tolerance in several species including rice [49], while other reports suggest that proline accumulation might mainly be a consequence of salt stress rather than being involved in its alleviation. In our study salt stress caused an increase in proline contents in both roots and shoots of rice seedlings. However, Si treatment caused a decrease in proline levels under salt stress compared to the seedlings treated with NaCl alone. Proline is considered to be a source of carbon and nitrogen for rapid recovery from stress and growth, a stabilizer for membranes and some macromolecules and also a free radical scavenger [47]. Tuna *et al*., (2008) [50] also reported decrease in proline contents in wheat cultivars grown under salinity and silicon and suggested that there is a definite decreasing effect of Si on proline accumulation. However, proline is a plant osmoregulator under stress condition and may cause decrease in growth and dry matter accumulation in salt stress [51]. Accumulation of proline under stress is a defensive mechanism in plants [52]. Proline protects enzymes maintain protein synthesis and plant tolerance against stress by several mechanisms such as removal of hydroxyl radicals and osmotic adjustment [53].

The present results indicate the increase of soluble reducing sugar and non reducing sugar content by NaCl treatment. Increased accumulation of sugars has been reported in many plant species exposed to salinity [35] [40] [54]. Accumulation of sugars has been related with drought and salinity tolerant mechanisms in many plant species. Hanafy Ahmed *et al.* (2002) [55] working on wheat mentioned that salinized plants accumulate soluble carbohydrate, amino acids, soluble phenols and proline for osmoregulation that allows the plants to maximize sufficient storage reserves to support basal metabolism under stressed environment [56]. Accumulation of soluble sugars helps to regulate osmotic stress in plant cells and leads to protection of biomolecules and membranes [57]. Therefore, metabolic alterations by sugar accumulation could contribute to salt sensitivity that limits growth of the rice seedlings under salt stress conditions. Hanafy Ahmed *et al.* (2008) [58] also demonstrated that soluble sugar contents of wheat plants decreased by adding silicon under saline soil conditions.

A decrease in starch contents was observed in rice plants under NaCl stress. Dubey and Singh (1999) [35] obtained the similar kind of results and concluded that the starch contents was reduced with higher magnitude in salt sensitive rice cultivars than salt tolerant cultivars under salinity stress. Starch is an important component of plant tissues and accumulates in leaves as a temporary reserve form of carbon and is the principal component of dry mass accumulated in mature leaves. Although starch may not play an important role in salt-tolerance mechanism, it is suggested that the ability of plants to partition sugars into starch may help to avoid metabolic alterations by lowering feedback inhibition caused by excess amount of sucrose in cytoplasm [59]. Another reason for reduction in starch concentration in plant tissue is the direct effects of decreased CO_2 assimilation caused by reduction in stomatal conductance and chlorophyll contents in plant tissues under salt stress [60]. Reduction of starch can be decomposed into smaller units that cause the accumulation of soluble sugars in plant cells [61]. Decrease in starch contents and increase in reducing and nonreducing sugar content were noted in leaves of *Bruguiera parviflora* under NaCl stress [62].

Sucrose phosphate synthase (SPS) is the major enzyme which catalyses the synthesis of sucrose phosphate during the last step of dark CO_2 fixation in photosynthetic and non-photosynthetic plant tissues [63] and is an important control point in biosynthesis of sucrose [64] [65]. In spinach leaves stimulation of SPS activity was observed under prolonged water stress [66]. The activity of SPS is also induced under low temperature and osmotic stress [65] and salinity [35]. Sucrose contents were found to increase in tomato (*Solanum lycopersicum*) under salinity due to increased activity of SPS [67]. The present study reveals the increased activity of SPS with NaCl treatment that finds support in the increase in non-reducing sugar like sucrose as reported earlier. But silicon treatment along with NaCl is found to ameliorate the effect of NaCl on SPS activity along with the decrease of sucrose contents in rice seedlings. The observed increase in sucrose contents is a specific response to salt, and not simply a result of decreased sucrose utilization, is supported by the observation that the activity of the sucrose synthesizing enzyme SPS is induced by salt stress [68] [69].

Starch phosphorylase is a starch degrading enzyme that catalyzes reversible phosphorylation of α-glucans producing glucose-1-phosphate and degrades starch beginning at a non-reducing end by incorporating phosphate [21]. The present result shows the increase in starch phosphorylase activity under increased concentrations of NaCl treatment. This increase in starch phosphorylase activity is correlated with the decreased starch contents in both roots and shoots of the test cultivar. Similar result was reported in rice [35]. But the activities of the enzyme reversed with the application of silicon along with NaCl. This causes decrease in starch phosphorylase activity and the level of starch contents was found to be increased. Increased activity of starch phosphorylase under salinity might help starch degradation and mobilisation of sugars.

Sucrose is the major carbohydrate imported by many plant sink tissues. Since sucrose is not the direct substrate for most of the metabolic processes involved in growth, development and storage in most sinks, conversion of sucrose to hexoses often is the primary starting point for sink metabolism [70] [71]. Huber and Akazawa (1986) [72] presented the theory of two different pathways for sucrose degradation, one carried out by sucrose synthase (SS) and other by invertase. Sucrose synthase is a cytosolic enzyme that catalyzes sucrose breakdown in vivo [63]. Acid invertase is located in the vacuoles and catalyzes hydrolysis of sucrose to glucose and fructose [73]. These enzymes play an important role in phloem loading and unloading by maintaining sucrose concentration gradient [74]. During anoxia of rice plants, there is an increase in the activities of SS [75]. Decrease in acid invertase activity was noted in rice plants under salinity [35] [40] and in *Phaseolus vulgaris*, salt stress causes inhibition of acid invertase synthesis [76]. The present results also show the increase in sucrose synthase activity and decrease in acid invertase activity under NaCl treatment. The results also indicate that decrease in acid invertase activity does not affect the reducing sugar contents which are mainly controlled by sucrose synthase activity. But the activities of these enzymes reversed with the application of silicon along with NaCl.

5. Conclusion

Our study demonstrates that application of NaCl in growing rice seedlings induced accumulation of proline, H2O2, MDA, reducing and non-reducing sugars with a concomitant increase in the activities of starch phosphorylase, sucrose phosphate synthase, sucrose synthase and decrease in the activities of catalase and acid invertase. Silicon may act to alleviate NaCl induced stress in rice seedlings which is evident from the morphological and biochemical studies. It seems that co-application of Si increased antioxidant activities and inhibited lipid peroxidation of cell plasma membranes to maintain integrity in high level of Na^+ concentration in the cytoplasm.

Therefore, the use of silicon enriched fertilizers in NaCl prone soils may help to improve the sugar metabolizing activity leading to better growth and development of rice plants.

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